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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61B 5/055

A1

(11) International Publication Number: **WO 99/52428**

(43) International Publication Date: 21 October 1999 (21.10.99)

(21) International Application Number: PCT/US99/07664

(22) International Filing Date: 8 April 1999 (08.04.99)

(30) Priority Data:
60/081,488 13 April 1998 (13.04.98) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**
*With international search report.*

(54) Title: *IN VIVO* MAGNETIC RESONANCE VASCULAR IMAGING USING LASER–POLARIZED GAS MICROBUBBLES

(57) Abstract

Nuclear magnetic resonance (NMR) images of a human or animal subject's vascular system are enhanced by injecting a liquid comprised of a biocompatible liquid carrier and a dispersion of hyperpolarized gas microbubbles into the subject, followed by generating an image by NMR representing a spatial distribution of the hyperpolarized gas microbubbles injected into the human or animal subject's vascular system. Preferably, the hyperpolarized gas in Helium–3 and/or Xenon–129. The microbubbles most preferably have a mean diameter of less than about 35 $\mu$m.

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# *IN VIVO* MAGNETIC RESONANCE VASCULAR IMAGING USING LASER-POLARIZED GAS MICROBUBBLES

## FIELD OF THE INVENTION

The present invention relates generally to magnetic resonance imaging (MRI), and more specifically, to MRI techniques using laser-polarized gases. In preferred forms, the present invention is embodied in techniques whereby injectable liquid suspensions of laser-polarized gas microbubbles are employed to enhance vascular magnetic resonance images.

## BACKGROUND AND SUMMARY OF THE INVENTION

High signal magnetic resonance (MR) images of void spaces, notably the lungs, have been acquired using laser-polarized or hyperpolarized noble gases, such as Xenon-129 and/or Helium-3. (See, U.S. Patent No. 5,545,396, the entire content of each being expressly incorporated hereinto by reference, and References 1-4). Xenon-129 has also been used as a probe for blood, muscle, and brain tissue. (References 5-7). These studies rely on xenon dissolving in a carrier, such as lipid vesicles or blood. Since helium is 10-100 times less soluble than xenon in such materials (Reference 8), Helium-3 has been used exclusively for imaging air spaces. However, considering that the signal of Helium-3 is over 10 times greater than that of Xenon-129 for presently attainable polarization levels, it would be highly desirable to discover some means by which Helium-3 could be introduced into the vascular system. According to the present invention, such means are provided.

Broadly, the present invention is embodied in introducing a hyperpolarized noble gas (e.g., Helium-3 and/or Xenon-129) into a human or animal vascular system in the form of an injectable liquid containing microbubbles of the hyperpolarized gas dispersed or suspended in a biologically compatible carrier liquid. The techniques of the present invention allow for a potential increase in

signal, and absence of background thereby permitting high resolution MR images to be obtained of human or animal vascular systems (i.e., angiographic images).

These and other aspects and advantages of the present invention will become more clear after careful consideration is given to the detailed description of the preferred exemplary embodiments thereof which follow.

## BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Reference will hereinafter be made to the accompanying FIGURES wherein,

FIGURE 1 is a graph of the approximate size distribution of Helium-3 microbubbles suspended in a conventional radiographic contrast agent;

FIGURES 2a and 2b are MR images of rat pelvic arteries and veins, respectively, obtained using Helium-3 microbubbles; and

FIGURE 3a is a Helium-3 MR image of a rat abdominal aorta and renal arteries, and FIGURE 3b is a $^{1}H$ image acquired with a standard time-of-flight angiographic pulse sequence for comparison against the image of FIGURE 3a.

## DETAILED DESCRIPTION OF THE INVENTION

The injectable hyperpolarized gas suspensions of this invention necessarily contain a biocompatible liquid carrier and microbubbles of a hyperpolarized noble gas. In this regard the carrier may be virtually any biocompatible liquid medium possessing minimal (if any) pharmacological effects and capable of containing sufficient amounts of the hyperpolarized gas microbubbles to produce the necessary SNR for *in vivo* MR imaging. Thus, according to the present invention conventional radiographic contrast agents (e.g., Hexabrix®, Renografin®, Isovue® and Omnipaque® contrast agents) and plasma volume expanders (e.g., Dextran and Heastarch) may be employed as the liquid carrier. When employing Helium-3 as the hyperpolarized gas, it is presently preferred to employ Hexabrix® radiographic contrast agent commercially available from Mallinckrodt Medical

since it exhibits higher relative SNR (i.e., 1.00 ± 0.19) as compared to the other carriers. In this regard, the preferred carrier is an iodine-based mixture containing ioxaglate meglumine (approx. 39.3%) and ioxaglate sodium (approx. 19.6%), and a minor amount of edetate calcium disodium, and exhibits a viscosity of about 15.7 cP at 20°C, a specific gravity of about 1.350 and a pH of 6-7.6.

The noble gas that is employed in the present invention is selected from noble gas isotopes having nuclear spin, preferably Helium-3 and/or Xenon-129. The noble gas may be hyperpolarized by any conventional technique (U.S. Patent No. 5,642,625, the entire content of which is expressly incorporated hereinto by reference, and References 2, 9 and 10). Thus, the gas that is employed in the present invention is preferably one capable of being hyperpolarized by optical (laser) pumping in the presence of an alkali metal or by metastability exchange. That is, valence electrons in a Rb vapor may be optically pumped with circularly polarized laser light. Through collisional spin exchange, angular momentum is transferred to the noble gas nuclei.

Preferably, the injectable microbubble suspensions in accordance with the present invention are formed by introducing a previously hyperpolarized noble gas into a suitable carrier liquid and then vigorously agitating the mixture to form microbubbles suspended in the carrier liquid. The agitation that is employed is of sufficient intensity to achieve a mean microbubble diameter of less than about 35 μm, and usually about 32 μm. Most preferably, the mean microbubble diameter will be less than about 10 μm, and typically less than about 8 μm, which is approximately the size of a blood cell. Such a size will allow the safe passage of the microbubbles through the pulmonary circulation (Reference 13). According to Stokes' Law, characteristics of the suspending liquid carrier, including surface tension, density, and viscosity, affect the microbubble size distribution and the rate at which the microbubbles rise to the surface. The size distribution of the microbubbles suspended in Hexabrix® contrast agent was determined using a Coulter Counter (Figure 1).

The concentration of hyperpolarized gas microbubbles in the liquid carrier is sufficient to allow MR signal acquisition over the time that imaging is conducted. To enhance image quality, it is essential to preserve the $^3$He polarization while the microbubbles are suspended and prepared for injection. Due to its nonequilibrium nature, magnetization decays with a characteristic time (longitudinal relaxation time or $T_1$) that depends on its surrounding environment. Additionally, signal loss occurs if the microbubbles physically rise out of the liquid carrier before injection of the suspension. Measurement of the combined effects of depolarization and rising bubbles in a phantom yielded an effective decay time of 41.6 ± 8.7 seconds for Hexabrix® radiographic contrast agent. This value is a lower limit of the actual $T_1$ and indicates adequate magnetization will persist throughout the mixing and delivery process. The time constant for the decay of the MR signal obtained from each radio frequency (RF) excitation is known as $T_2^*$. By measuring the linewidth of the Fourier transformed signal in the $^3$He bubbles, it was found that $T_2^* \cong 20$ ms.

A further understanding of this invention will be obtained from the following non-limiting Examples.

## EXAMPLES

$^3$He was polarized to 10-15% by the spin exchange method (Reference 2). 2 cm$^3$ of $^3$He was withdrawn into an evacuated 10 cm$^3$ plastic syringe. This syringe was then connected, via a plastic three-way stopcock, to a second 10 cm$^3$ syringe containing 8 cm$^3$ of liquid carrier. Rapidly flushing the fluids several times between syringes produced a suspension of $^3$He microbubbles.

Imaging was performed on a 2.0 T, 30-cm-bore Oxford magnet with shielded gradients using a GE signa console and a 7-cm-diameter dual-frequency ($^3$He and $^1$H) birdcage coil.

*In vivo* imaging was performed with male Sprague-Dawley rats (400 - 480 g) that were anesthetized with either pentobarbital sodium or isoflurane. For venous injections, a 22-gauge plastic cannula was inserted into a lateral tail vein, whereas for arterial injections, a catheter (PE 50 tubing) was inserted into the

aorta via the carotid artery. Immediately after creating the $^3$He microbubble suspension, 7 cm$^3$ were injected over a period of either 10 or 26 seconds during which imaging occurred.

The animal was placed in the supine position inside a dual-frequency, 7-cm-diameter birdcage RF coil operating at 64.8 MHz and 85.5 MHz for $^3$He and $^1$H, respectively. All $^3$He imaging employed a standard 2D gradient-recalled echo pulse sequence (Reference 20) with the following parameters: 79 mm field-of-view (FOV), 128 × 256 matrix size zero-filled to 256 × 256, and 1.2 ms effective echo time (TE). Both images in Figures 2a and 2b used an 80 ms repetition time (TR) and 20° flip angle ($\alpha$), while Figure 3a was obtained with a 200 ms TR and 15° $\alpha$. The 3D $^1$H image (Figure 3b) was acquired using a vascular time-of-flight sequence (Reference 15) with 79 mm FOV, 192 × 256 matrix size zero-filled to 256 × 256, 2.2 ms TE, 18 ms TR, 30° $\alpha$, 2 excitations, and 0.7 mm slice thickness. RF pulses associated with image acquisition necessarily depolarized the $^3$He. As a result, the RF power (i.e., flip angle), repetition time, and injection rate were carefully chosen to ensure that sufficient magnetization would remain throughout the region of interest (References 13-14).

Images of both the arteries and veins of the rat pelvic region obtained using $^3$He microbubbles suspended in Hexabrix® are shown in accompanying Figures 2a and 2b, respectively. Excellent delineation of all major vessels can be seen, with a maximum SNR ≅ 55 in both images. Observable blood vessels include the abdominal aorta, common iliac, and external iliac arteries in Figure 2a, and the vena cava, common iliac, and caudal veins in Figure 2b.

A $^3$He image of the abdominal aorta and renal arteries is shown in Figure 3a. In the lower portion of this image, a faint line runs parallel with the aorta. It is believed that the faint line is an image of the vena cava, based on the anatomy depicted in a corresponding proton image acquired with a standard time-of-flight angiographic pulse sequence (Figure 3b) (Reference 15). This means sufficient amounts of polarized $^3$He bubbles reached the venous circulation. The absence of

the vena cava in Figure 2a is probably a result of depolarization caused by using a larger flip angle and a shorter repetition time.

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While the invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

## ADDENDUM OF REFERENCES

(The entire content of each reference cited below is expressly incorporated hereinto by reference.)

1. Albert, M.S. *et al.* Biological magnetic resonance imaging using laser-polarized $^{129}Xe$, *Nature* **370**, 199-201 (1994).

2. Middleton, H. *et al.* MR imaging with hyperpolarized $^{3}He$ gas, *Magn. Reson. Med.* **33**, 271–275 (1995).

3. Black, R.D. *et al.* In vivo He-3 MR images of guinea pig lungs, *Radiol.* **199**, 867-870 (1996).

4. Wagshul, M. *et al. In vivo* MR imaging and spectroscopy using hyperpolarized $^{129}Xe$, *Magn. Reson. Med.* **36**, 183-191 (1996).

5. Bifone, A. *et al.* NMR of laser-polarized xenon in human blood, *Proc. Natl. Acad. Sci. USA* **93**, 12932-12936 (1996).

6. Goodson, B.M. *et al. In vivo* NMR and MRI using injection delivery of laser-polarized xenon, *Proc. Natl. Acad. Sci. USA* **94**, 14725-14729 (1997).

7. Swanson, S.D. *et al.* Brain MRI with laser-polarized $^{129}Xe$, *Magn. Reson. Med.* **38**, 695-698 (1997).

8. Weathersby, P.K. & Homer, L.D. Solubility of inert gases in biological fluids and tissues: a review, *Undersea Biomed. Res.* **7**, 277-296 (1980).

9. Happer, W. *et al.* Polarization of the nuclear spins of noble-gas atoms by spin exchange with optically pumped alkali-metal atoms, *Phys. Rev. A* **29**, 3092–3110 (1984).

10. Chupp, T., Wagshul, M., Coulter, K., McDonald, A. & Happer, W. Polarized high-density gaseous $^{3}He$ targets, *Phys. Rev. C* **36**, 2244–2251 (1987).

11. Saam, B., Happer, W. & Middleton, H. Nuclear relaxation of $^{3}He$ in the presence of $O_2$, *Phys. Rev. A* **52**, 862-865 (1995).

12. Tei, C. *et al.* Myocardial contrast echocardiography: a reproducible technique of myocardial opacification for identifying regional perfusion deficits, *Circulation* **67**, 585-593 (1983).

13. Johnson, G.A. *et al.* Dynamics of magnetization in hyperpolarized gas MRI of the lung, *Magn. Reson. Med.* **38**, 66-71 (1997).

14. Chen, X.J. *et al.* MR microscopy of lung airways with hyperpolarized $^{3}$He, *Magn. Reson. Med.* **39**, 79-84 (1998).

15. Dumoulin, C.L., Cline, H.E., Souza, S.P., Wagle, W.A. & Walker, M.F. Three-dimensional time-of-flight magnetic resonance angiography using spin saturation, *Magn. Reson. Med.* **11**, 35-46 (1989).

16. Feinstein, S.B. *et al.* Microbubble dynamics visualized in the intact capillary circulation, *J. Am. Coll. Cardiol.* **4**, 595-600 (1984).

17. Bachert, P. *et al.* Nuclear magnetic resonance imaging of airways in humans with use of hyperpolarized $^{3}$He, *Magn. Reson. Med.* **36**, 192-196 (1996).

18. Winkelmann, J.W., Kenner, M.D., Dave, R., Chandwaney, R.H. & Feinstein, S.B. Contrast echocardiography, *Ultrasound in Med. & Biol.* **20**, 507-515 (1994).

19. Sodee, D.B. *et al.* in Principles and Practice of Nuclear Medicine (eds. Early, P.J. & Sodee, D.B.) 370-442 (Mosby, St. Louis, 1995).

20. Glover, G.H. & Pelc, N.J. A rapid-gated cine MRI technique, *Magn. Reson. Annual* 299-333 (1988).

21. Chawla, M.S. *et al.* The feasibility of vascular MR microscopy using hyperpolarized gas, Proc. ISMRM, 5th annual meeting, Vancouver, p. 2114 (1997).

**WHAT IS CLAIMED IS:**

1. A method of nuclear magnetic resonance (NMR) imaging of a vascular system of a human or animal subject comprising:

(a) injecting a liquid comprised of a biocompatible liquid carrier and a dispersion of hyperpolarized gas microbubbles into the human or animal subject's vascular system; and then

(b) generating an image by NMR representing a spatial distribution of said hyperpolarized gas microbubbles injected into the human or animal subject's vascular system.

2. The method of claim 1, wherein the hyperpolarized gas is a noble gas.

3. The method of claim 2, wherein the hyperpolarized noble gas is Helium-3 and/or Xenon-129.

4. The method of claim 1, further comprising prior to step (a), the step of mixing the liquid carrier and an NMR effective amount of the hyperpolarized gas under sufficient agitation conditions to achieve microbubbles of said hyperpolarized gas suspended in said liquid carrier.

5. The method of claim 1, wherein prior to said mixing step, there is practiced the step of subjecting a non-polarized noble gas to spin-exchange polarization to achieve a hyperpolarized noble gas.

6. The method of claim 5, wherein said step of subjecting the non-polarized noble gas to spin-exchange is practiced such that the noble gas is polarized between about 10% to about 15%.

7. The method of claim 1, wherein the microbubbles have a mean diameter of less than about 35 μm.

8. The method of any one of the preceding claims, wherein the method is practiced *in vivo* with a human or animal subject.

9. A biocompatible injectable liquid to enhance nuclear magnetic resonance (NMR) images of human or animal vascular systems comprising:

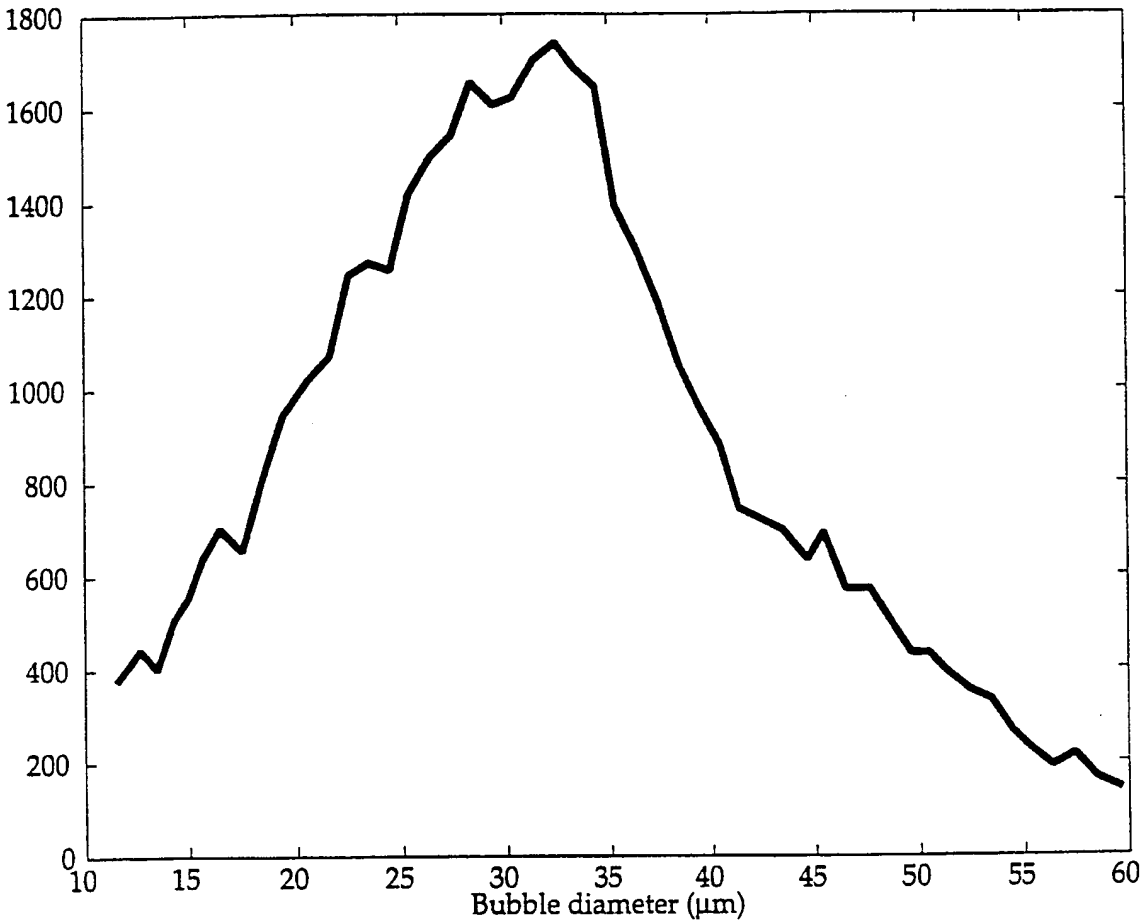
(a) a biocompatible liquid carrier; and

(b) a NMR enhancing effective amount of hyperpolarized noble gas microbubbles suspended in said liquid carrier.

10. The injectable liquid of claim 9, wherein the hyperpolarized noble gas is Helium-3 and/or Xenon-129.

11. The injectable liquid of claim 9 or 10, wherein the hyperpolarized noble gas microbubbles have a mean diameter of less than about 35 μm.

12. The injectable liquid of claim 9 or 10, wherein the hyperpolarized gas is spin-polarized between about 10% to about 15%.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Bubble diameter (µm)

Fig. 1

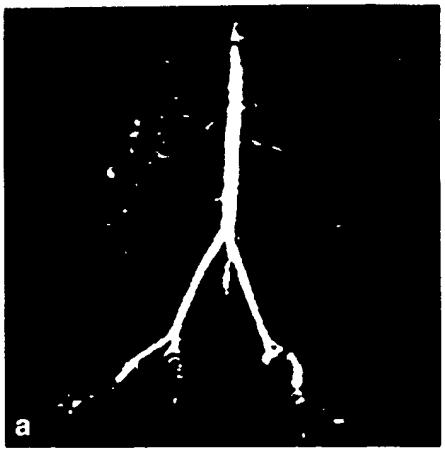


Fig. 2A

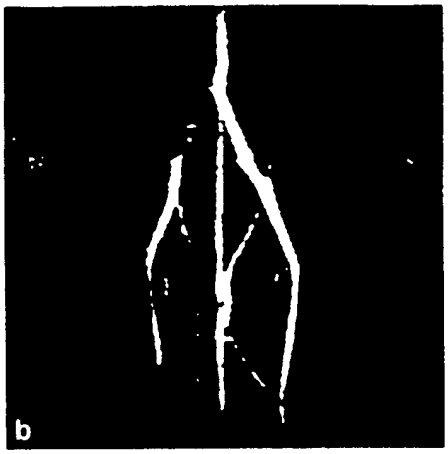


Fig. 2B


Fig. 3A


Fig. 3B

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/07664

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61B 5/055
US CL :424/9.3; 600/420
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.3; 600/420

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, MEDLINE
search terms: hyperpolarized, magnetic resonance imaging

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5,545,396 A (ALBERT et al.) 13 August 1996, col. 12, lines 4-11 and lines 44-55. | 1-12 |
| A | US 5,642,625 A (CATES, Jr. et al.) 01 July 1997. | 1-12 |

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